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13. ABSTRACT (Maximum 200 words)

A robust and effective model for determining the presence or absence of an analyte in a DPN-assembled gold nanoparticle/DNA conjugate structure in the limit of single molecule binding was developed. The capacitance picture of semi-classical mesoscopic transport provides both greater sensitivity and greater reproducibility than focusing on the charge transport within the DNA. Effectively, the capacitance model works well in the coulomb blockade limit that is relevant for such sensing structures, and gives higher reliability and sensitivity than relying on changes in the conductance of the DNA strands themselves. The basic components of these sensors are nanoparticles functionalized with biological markers (DNA or protein) for bio-warfare agents as well as nanoelectronic systems for signal transduction. Methodologies were developed in our laboratories to fabricate tools for patterning proteins and DNA structures using custom fabricated cantilever arrays by the industrial partner NanoInk using a single feedback system. Patterning of nanostructures was achieved through the use of custom pens and inkwell systems. Nanoparticle based screening methods for multiplexed DNA assays were developed by the industrial partner Nanosphere and a multiplexed assay for six select agents was demonstrated via micro array as well as the novel bio-barcode assays. Antibody sandwich assays using DPN template patterning were demonstrated using metal mediated coordination chemistry.

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FINAL REPORT

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Ultrasensitive, Ultradense Nanoelectronic Biosensing with Nanoparticle Probes

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SUMMARY

This report summarizes major scientific and technological accomplishments during the first phase of the MoleApps program. It includes an extensive list of articles published in peer reviewed journals and participation in scientific meetings, workshops, and lectures. Finally, patent disclosures, transitions, and awards earned by the participating investigators are listed. The MoleApps program was exceptionally productive as can be deduced from the highlights of the scientific and technological breakthroughs in the following paragraph.

The focus of our effort was in three major areas:

A - Theoretical understanding of devices at the nanoscale

B - Nanofabrication and nanopatterning to generate these devices

C - Assay development and biological screening to address nanogaps

Significant progress was made toward theoretical and experimental understanding of biosensors at the nanometer length scale. The code for a robust and effective capacitance model for deducing the presence or absence of an analyte through a molecular recognition event was developed. This model showed that at the limit of a single binding event generated through the Dip-Pen Nanolithography (DPN) assembled gold nanoparticle/DNA conjugate, greater sensitivity and reproducibility may be achieved. The capacitance model works well in the coulomb blockade limit relevant for such sensors.

In the nanofabrication and nanopatterning area, NanoInk successfully fabricated a million pen array of cantilevers, and robust patterning with at least 25000 pens over a square centimeter area was demonstrated. Control over device gap size was demonstrated through a novel technique, On-Wire Lithography (OWL) with exceptional simplicity in methodology. DPN-based methods for the generation of nanoelectrodes were developed and successfully used for patterning of biomolecules onto electrode gaps at 100-nm resolution. The detection of DNA in solution was demonstrated using a nanoparticle/nanoelectrode sensor with Ohmic response.

In the assay development and biological screening area, our industrial partner, Nanosphere Inc., designed a simple and robust nanoparticle based DNA assay for select bio-warfare agents. This assay methodology was then multiplexed to detect six human pathogens in a multiplexed format. Concurrent with Nanosphere's work a multiplexed bio-barcode assay for DNA targets with exceptional sensitivity down to the low femtomolar concentration range was also developed.

While the majority of important milestones was reached, as well as exceeded during phase I, future challenges remain. These challenges include the fabrication and further miniaturization of multi-component arrays, further design and development of multiplexing levels, protein-based particle probes, and enhanced registration capability using 2-D arrays, as well as more efficient inking formulations and procedures for biomolecule patterning. Finally, analytical benchmarking of the sensitivity and selectivity of these nanosensor devices needs to be addressed in future experiments. With regard to other accomplishments, many postdoctoral associates and graduate students were trained in areas of vital importance to the mission of the DOD, with potential payoff for the agency in the near future, and the long-term deployment of many moletronic applications. The highlights of our accomplishments are featured in the following report.

1. OBJECTIVES

The objectives of this interdisciplinary project were to develop both a theoretical as well as an experimental understanding of ultra-sensitive, ultra-dense nanoelectronic biosensors.

2. STATUS

A robust and effective model for deducing the presence or absence of an analyte in a DPN-assembled gold nanoparticle/DNA conjugate structure in the limit of single molecule binding was developed. The capacitance picture of semi-classical mesoscopic transport provides both greater sensitivity and greater reproducibility than focusing on the charge transport within the DNA. Effectively, the capacitance model works well in the coulomb blockade limit that is relevant for such sensing structures, and gives higher reliability and sensitivity than reliance on changes in the conductance of the DNA strands themselves.

The basic components of these sensors are nanoparticles functionalized with biological markers (DNA or protein) for bio-warfare agents as well as nanoelectronic systems for signal transduction. Methodologies were developed in our laboratories to fabricate tools for patterning proteins and DNA structures with custom fabricated cantilever arrays developed by our industrial partner NanoInk using a single feedback system. Patterning of nanostructures was achieved through the use of custom pens and inkwell systems. Nanoparticle-based screening methods for multiplexed DNA assays were developed by our industrial partner Nanosphere and a multiplexed assay for six select agents was established using microarrays as well as the novel bio-barcode assays. Antibody sandwich assays using DPN template patterning were developed using metal mediated coordination chemistry.

ACCOMPLISHMENTS/NEW FINDINGS

A. Theoretical Model (Ratner, Mirkin)

Complete model understanding of transport in gold nanoparticle/quantum dot structures assembled following DNA target recognition using DPN.

Using nanoelectronics for biosensing of DNA or protein targets, one could imagine two different approaches. In the first one, differences in conductance of individual DNA double helix structures arising from changes in the sequence might be utilized. The second approach would be to use the five-component structure electrode/DNA/gold nanodot/linker/electrode, and to analyze the resulting circuit in the quantum dot limit, using the classical capacitance model. Each of these two approaches has advantages: the conductance variations are really quite sensitive to SNP modifications, while binding (particularly if the target strand is a long one) is less sensitive to such structures. The binding assay, however, is advantageous because the shape of the conductance curve is really very well fit using the classical capacitance model. Therefore the analysis has its own self-correcting scheme for errors. If the line shape does not fit the capacitance model, there has been some form of mis-assembly, and the finding should be rejected. Such behavior is not found in the simple conductance picture, where only an overall scale factor differentiates between the perfect conjugate and an SNP.

In deriving these conclusions, three activities in pursuit of the objectives and milestones in the original proposal have been carried out. First, our studies of the conductance of DNA strands, their dependence on sequence, and their sensitivities to SNPS have essentially been completed. These results largely confirm previous work, but the actual extent of the modifications due to solvation, dynamics, salt, and other variables has been clarified. Our general picture of conductance by combination of hopping and tunneling seems to be in agreement with a very large amount of experimental data, as well as modeling studies from other laboratories.

Second, the formal aspects of calculating conductance in the quantum dot limit appropriate for the nanoparticle/DNA probe employed by the Mirkin group were investigated. The standard non-equilibrium Greens function/density functional theory approach seems to fail badly in this limit, as might have been anticipated by the analogy to the so-called super molecular limit in molecular electronic structure calculations. Work is ongoing in this regard, because this limit is of fundamental importance to our understanding of molecular transport junctions both for sensing and further applications. Nevertheless, our studies indicate that utilizing the capacitance limit is a substantially more attractive and useful approach than is modification of the strand conductance itself.

Finally, our molecular dynamics studies, Monte Carlo studies, and scaling studies of how DPN assembly actually works have been extended. This is important, because in the actual sensing applications the DPN-created structures will vary in their geometries. Once again, it was found that for the appropriate low concentration limit, the DPN-deposited gold dot conjugate structures are really quite robust. Modifications of the line shape of the conductance/voltage measurement do indicate when misalignment has occurred, and such structures can be rejected.

We are pleased with the modeling studies that have been completed under this grant, and believe that the accuracy, viability and self-correcting character of a quantum dot probe scheme developed by the Mirkin group and extended by both Nanosphere and NanoInk has been shown. While a great deal of modeling remains to be done (e.g. extension to possible protein analysis, analysis of the dilute limit in which structural errors may become more frequent, analysis under other non-optimal conditions of solvation or temperature, interference analysis in very dense structures), the modeling activity, combined with the quite remarkable advances in DPN-based structure and transport analysis, does support the robustness and ultra-sensitivity of the nanoparticle conjugate DNA probe.

B. Nanofabrication and Nanopatterning (Mirkin, NanoInk Inc.)

For phase I of this project, NanoInk fabricated custom DPN pens and inkwells needed for nanoscale patterning in oligonucleotide and protein arrays. Because these inks are viscous and sticky, the pens need to be much stiffer so that they can be released from the inkwells and the surface after writing. The design chosen was to make the silicon nitride cantilever material thicker; a first for silicon nitride cantilevers. The alternative, making the pens shorter, would not work with the laser optics for the feedback system on the Nscriptor™. In order for the inkwells to feed the DNA pens, a new more hydrophobic top surface had to be developed to prevent the DNA ink from spilling out of the wells. The previous surface had a contact angle with water about the same as Teflon or 108°. The new hydrophobic coating was greatly improved with a contact angle of >140°. In

addition, design features called cliffs were added around each inkwell to eliminate the possibility of inks from adjacent wells mixing on the surface between the wells. To reduce the potential for evaporation of the inks, the reservoirs, channels and wells were etched much deeper into the silicon.

To pattern large areas quickly a 2-D pen array was conceived. NanoInk fabricated a 25,000 pen array using similar technology as for the 1-D array used previously. A method for scribing the Pyrex glass before the silicon mold wafer was etched away was developed. After fabrication of the wafers, the chips were snapped into 1 cm squares. For use in the Nscriptor, the 2-D array chip was placed on the substrate and a dot of glue placed on its back. A standard DPN pen chip, with the probe removed, was brought down into the glue, and the chip was brought down to allow a rudimentary two axis passive alignment.

High-throughput Lithography via DPN

This work addresses the issue of increasing the throughput of DPN, which is a challenge facing all scanning probe lithographies, and is of importance for a wide variety of fields, especially nanoelectronics and biological nanoarrays that require large arrays of sub-100 nm structures. Previous work has dealt with 1-D linear arrays of cantilevers operating in parallel and has demonstrated a throughput as high as 10^6 nanostructures/hr. Herein patterning using 2-D arrays of cantilevers is demonstrated (Figure 1). This may potentially allow for massive parallel lithography rivaling the throughput of photolithography, while still maintaining sub-100 nm resolution.

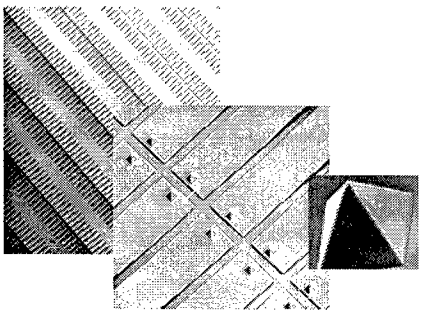


Figure 1. SEM images of 2D tip array.

As a proof-of-concept experiment, the tip array was vapor coated using ODT (65C°, 30 min), and then used for patterning onto a gold substrate (30 nm Au evaporated onto 10 nm Ti). The fidelity of patterns was investigated by selectively etching ($Fe(NO_3)_3$ + thiourea, etch rate 2 nm/min) unprotected regions of the gold substrate. Figure 2 shows the resulting gold structures. The distribution of gold dots matches the spacing of the 2-D array shown in Figure 2. This is the first demonstration of 2-D array patterning in the context of DPN, and indeed, square centimeter areas could be patterned in seconds. Future work entails implementing complex patterns and further improving tip design to minimize the amount of space between the tips.

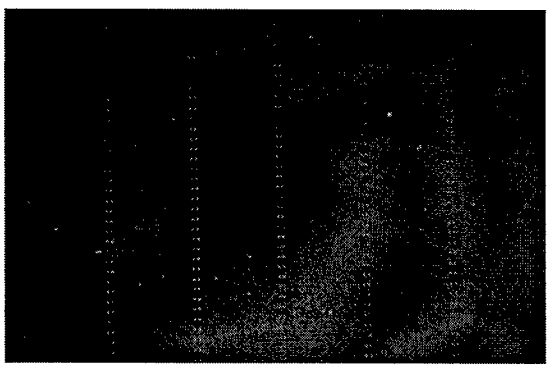
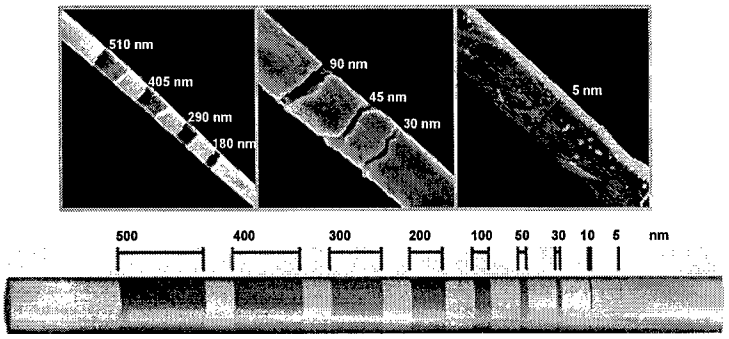


Figure 2. 2D array using DPN

On-Wire Lithography (OWL)

A high-throughput procedure was developed for lithographic processing of one-dimensional nanowires to generate nanowires with regular nanoscale gaps. This procedure, termed "On-Wire Lithography" (OWL), combines advances in template directed synthesis of nanowires with electrochemical deposition and wet-chemical etching, and allows one to routinely fabricate face-to-face disk arrays and gap structures in the 2 nm to several hundred nanometer range. In our procedure, striped metallic nanowires were synthesized, and then dispersed on a substrate. Sonication resulted in dispersion of the wires, and etching of sacrificial stripes formed gaps of the desired dimensions.

OWL is based on the idea that one can fabricate segmented nanowires consisting of at least two types of materials, one that is susceptible, and one that is resistant to wet-chemical etching. For proof-of-concept experiments Au-Ag and Au-Ni were used. The process involving Au-Ni nanowires is described in the following. These materials can be electrochemically deposited into porous alumina templates (pore diameter 360 nm) in a controlled manner from suitable plating solutions through well-established methods (Scheme 1). The length of each segment can be tailored by controlling the charge passed during the electrodeposition process. The segments are then released from the template by dissolution of the template through published procedures. The aqueous suspension of nanowires was cast on a glass microscope slide, pretreated with piranha solution to render it hydrophilic, and allowed to dry in a dessicator. A layer of silica (50 nm) was then deposited onto the nanowire-coated substrate by plasma-enhanced chemical vapor deposition. The substrate was immersed in ethanol and sonicated (VWR Ultrasonic Cleaner, MODEL 50T) for 1 min, which released the wires. The final step of the OWL process involves the selective wet-chemical etching of the Ni segments. Ni can be removed from the wires by treatment with concentrated HNO_3 (1 hour). This treatment generates nanowire structures with gaps that are precisely controlled by the length of the original Ni segments (Scheme 1).



Scheme 1. On-Wire Lithography (OWL)

Measurements of I-V curves show that the gaps within the nanowires are insulating (green line in Figure 3). The gaps within the nanowires can be functionalized with many materials in a site-specific manner using DPN. As proof-of-concept, DPN was used to deposit a mixture of polyethylene oxide and self-doped polypyrrole (PEO:PPy= 1:1 w/w) into the gap.

This approach allowed monitoring the device architecture in the active region, measuring the topography of the nanowires, and simultaneously functionalizing the nanowire gaps with molecule-based materials. An SEM image after modification of the gap with polymer shows the clear contrast between the clean gold surface and the polymer covered area including the gap (Figure 3, inset). After deposition of the polymer,

the I-V curves show a linear response from –1.0 to 1.0 V, characteristic of the conducting polymer (red line in Figure 3). The measured conductance of 1.1 nS is similar to the value of 9.6 nS determined by functionalizing 60 nm conventional nanoelectrode gaps fabricated in our lab by electron-beam lithography. There is no noticeable I-V hysterisis between the forward (from –1.0 to 1.0 V) and backward (from +1.0 to –1.0 V) + scans, and they are highly linear at room temperature, as is expected for a structure with an ohmic-like contact in a symmetric device configuration.

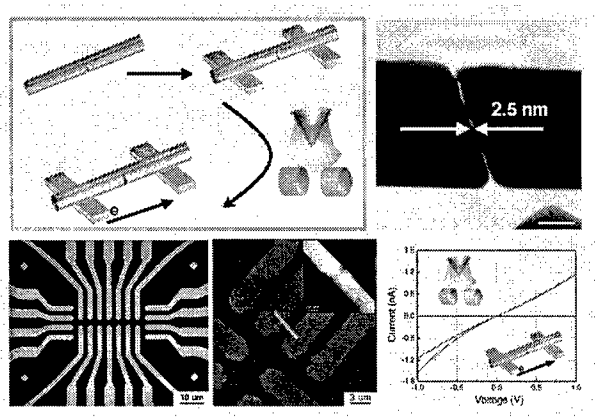


Figure 3. Electrical measurements in nanogaps

In order to show that the response is indeed from the polymer within the gap, we studied the I-V response as a function of photoexcitation with a Xe lamp (150 W). The I-V response for the polymer-filled nanowire became slightly more conductive upon Xe light exposure. During the backward scan when the device was irradiated with a Xe lamp starting at –0.1 V (red arrows in Fig. 3), a change in slope in the I-V response was observed. The transient conductance change between 1.1 nS in the dark and 1.6 nS when irradiated is consistent with an increase in charge-carrier density, which would be expected if the gap was filled with the p-type polypyrrole.

Being able to fabricate gaps or notched structures with nanowires using OWL and relatively inexpensive instrumentation will facilitate the study of the electronic properties of nanomaterials, and will also open avenues for the preparation of novel disk structures, which could be designed to display unusual optical properties as a function of gap and metal segment size. The DOD technology payoff will be general methods for fabricating device-directing templates for many moletronic applications.

C. Assay Development and Biological Screening (Mirkin, Nanosphere Inc.)

Nanosphere Inc. provided expertise in probe design, gold nanoparticle probe synthesis, assay development, and implementation of a multiplexed gold nanoparticle-based diagnostic chip assay for the MoleApps project. A simple multiplexed DNA assay was developed for six bio-warfare agents (*Bacillus anthracis*-anthrax, Variola virus, Ebola virus, HIV, and Hepatitis A and B viruses). DNA sequences for these agents were selected from public and private databases through computational methods. Based on these sequences, capture probes, targets, and gold probe sequences were designed and optimized for lack of secondary structure using thermodynamic parameters. The optimized sequences were chemically synthesized and the chip assay assembled on glass slides to test the selectivity and specificity of the target and capture probes.

Initial screening of these nanomaterials showed facile detection of the target sequences via the chip assay in the uniplex assay format. The results of the uniplex assays were then used to develop the uniplex assay into the multiplex assay format. The multiplexed chip assay was further refined and optimized with a single universal gold

nanoparticle probe designed for six different pathogens through a universal sequence tag for the target.

Multiplexed Bio-Barcode Assay for Bio-Weapons Targets

Oligonucleotide-functionalized gold nanoparticles (Au-NPs) have demonstrated a sensitivity and selectivity advantage over conventional probes in a variety of biodetection schemes. Their outstanding performance stems from cooperative binding, unique optical, and catalytic properties and the existence of robust and versatile surface functionalization methods. The bio-barcode assay, which is based upon Au-NPs functionalized with a large number of oligonucleotide strands (the barcodes) and a corresponding recognition agent (either an antibody in the case of protein targets or a portion of the barcode strand that can recognize the target of interest in case of nucleic acid targets), is particularly promising as it allows one to rapidly detect protein and nucleic acid targets at low-attomolar and high-zeptomolar concentrations, respectively, under optimized conditions. Significantly, targets can be detected without the need for enzymatic amplification of the target sequence prior to the detection event. In the bio-barcode assay, the barcode oligonucleotide strands are used as a target representative and as a means of amplification. For each target molecule recognized and captured in the sandwich assay, approximately 300 barcodes are released. Multiplexing is challenging for many assays because of the need to eliminate probe set/target set cross reactivity, minimize nonspecific binding, and design spectroscopically and

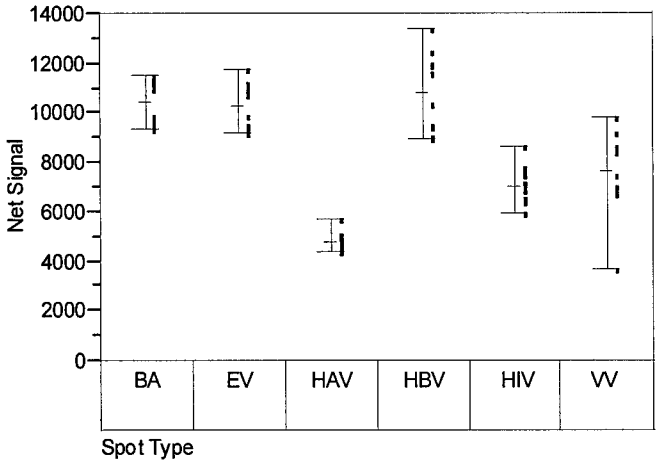


Figure 4. Net signal intensity vs. spot type in the multiplexed DNA assay using six targets Anthrax (BA), Variola (VV), Ebola (EB), HIV, Hepatitis A (HAV), and Hepatitis B (HBV)

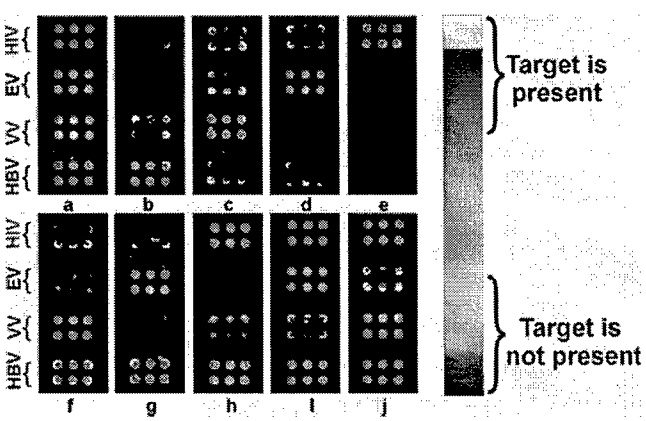


Figure 5. Scanometric detection of the barcode DNA strands released from the 30 nm Au NP probes for 10 different samples. (a) All targets are present. (b) HBV. (c) VV. (d) EV. (e) HIV. (f) HBV and VV. (g) HBV and EV. (h) HBV and HIV. (i) HBV, EV and HIV. (j) HBV, VV and HIV. The gray scale images from Verigene ID system are converted into colored ones using GenePix Pro 6 software (Molecular Devices).

chemically unique probes. The utility of the bio-barcode assay to detect multiple oligonucleotide targets simultaneously was demonstrated by using 30- to 33- nucleotide long sequences associated with (1) the Hepatitis B virus surface antigen gene (HBV), (2) the Variola virus (small pox, VV), (3) the Ebola virus (EV), and (4) the human immunodeficiency virus (HIV) as model systems. The barcode for each target is the sequence-specific nucleotide probe, while the reminder of the sequence is universal for scanometric detection and readout. It was shown that these four oligonucleotide targets can be detected with high selectivity at mid-femtomolar concentration. (Figure 5) Significantly, the multiplexed bio-barcode assay can be performed using a 96-well plate format in a high-throughput manner. This work shows the capability of oligonucleotide functionalized Au-NPs to recognize their cognate targets from a host of different sequences in a multiplexed manner, opening the door to parallel electrical detection using these same Au-NP probes.

Bio-Barcode Assay for Protein Targets

In addition to the bio-barcode work done for DNA multiplexing, this same assay has been applied to the multiplexed detection of protein targets. To date three different proteins have tested as targets in the multiplexed bio-barcode assay. Efforts to repeat the results in serum samples are currently being conducted.

Metal Ion Usage for Antibody Orientation by Dip-Pen Nanolithography (DPN)

An assortment of immobilization techniques has been tried for proteins, in particular antibodies, enzymes, and cell adhesion ligands. These techniques have mainly relied on the ability of proteins (protein A, G, A/G etc.) to bind selectively to the F_c region of antibodies which will leave the antigenic sites exposed.

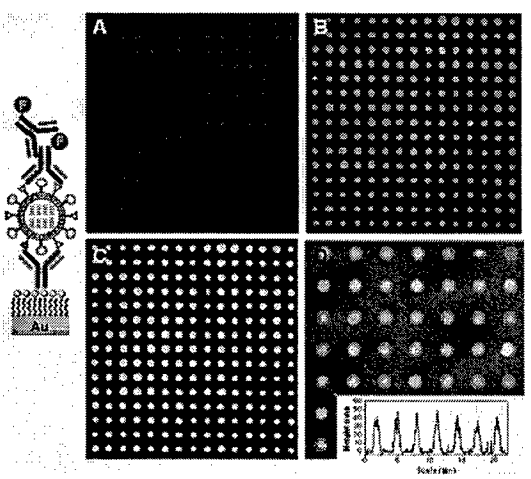


Figure 6. Fluorescent images of an MHA-Zn microarray (by -CP) subsequently exposed to anti-12CA5 (12CA5 is a specific sequence of HA region of influenza A (H3N2)) IgG1 antibody, influenza virus, anti-12CA5-Alexa Fluor 596 and goat anti-mouse Alexa Fluor-488. (A) Red, (B) Green, and (C) superposition of both fluorescent images. (D) AFM topographic image of the microarray. All AFM images were taken at a 0.5 Hz scan rate in tapping mode.

In addition, genetic engineering technologies have produced fusion protein-antibody conjugates with unusual binding tags for specific directed attachment, electrostatically driven adsorption, covalent linkage, or a combination of these, in order to arrange antibodies into their proper orientations and to maximize interactions between specific antigens. Considering the methodologies mentioned above, each one of these suffers from different drawbacks and limitations. A general strategy, which originated from the similar principle of Immobilized Metal Ion Affinity Chromatography (IMAC), was

developed. This approach used metal ions to chelate amino acids such as aspartic acid, glutamic acid, histidine, and cysteine around specific regions of antibodies and to direct optimal orientation for protein-target interactions. Only with our methodology can unlabeled, pristine antibodies be utilized directly without the expression of a histidine tag or any other protein modification strategies (Figure 6)

Recently, metal ion coordination chemistry in combination with lithographic techniques, such as micro-contact printing and DPN, has played a role in biotechnology for generating oligonucleotide microarrays and single virus nanoarrays. Metal ions have the advantage of being simple, robust, economical, versatile, capable of long-term storage, and resistant to denaturing effects that affect proteins used for antibody immobilization (i.e. protein A, G, A/G etc). Antibody immobilization on metallated surfaces is influenced by several factors, including electrostatics, van der Waals interactions, hydrophobic effects, pH and ionic strength.

A key principle of our method is to take advantage of the isoelectric points (IEP) of the different regions of the antibody. The carboxylic acids in the F_c fragment can be selectively deprotonated to form negatively charged carboxylates at pH < 6.5, while those of the F_{ab} regions have a significantly higher IEP, usually pH > 8. In PBS buffer at pH 7.4, the positively charged metal-ion layer can serve as an adhesive to facilitate the formation of coordination complexes with a negatively charged carboxylate rich F_c region. Thus, by using this method one can orient an antibody in an "end-on" position, in which the F_{ab} regions are exposed for optimal orientation of protein-target interactions. Herein the surface immobilization and orientation of several unmodified antibodies (mouse IgG1, goat IgG, rabbit IgG, and mouse IgM) in the context of μ-CP and DPN on metal-ion exposed arrays were studied. These antibodies were selected to cover the affinity spectrum that protein A, protein G, or recombinant protein A/G, have on binding to a solid support.

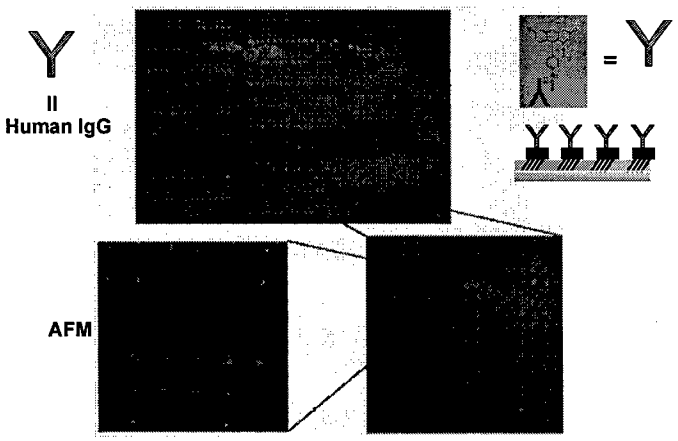


Figure 7. Direct-Write Antibodies

Direct Write Antibodies

Our previous work has demonstrated the capability of DPN to write proteins directly onto a surface (Figure 7). Though this method is unique and powerful, controlling the orientation of these patterned proteins, such as antibodies, is still in progress. It is anticipated that the technology payoff will be the assembly of highly miniaturized biosensing devices that are field deployable, and allow identification of bio-warfare as well as anti-terrorism agents without the use of PCR.

4. PERSONNEL SUPPORTED

Postdocs	Percentage of Salary Provided by Grant
Yu-Hsu Chang (Mirkin)	45 %
Ling Huang (Mirkin)	55 %
Sergey Rozhok (Mirkin)	75 %
Yuhuang Wang (Mirkin)	100 %
Eric Brown (Ratner)	5 %
Michael Galperin (Ratner)	5 %
Gil Katz (Ratner)	75 %
Vladimiro Mujica (Ratner)	40 %
Chad Risko (Ratner)	10 %
Baudilio Tejerina (Ratner)	45 %

Graduate Students:	Percentage of Salary Provided by Grant
David Andrews (Ratner)	10 %

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6. INTERACTIONS

A. Presentations/Presentations at Meetings, Conferences, Seminars

AFOSR Biomimetic Program, San Diego CA, 2005, "Ultrasensitive and Selective Chip Based Detection of DNA." (Mirkin)

Nanoscience Seminar Series, Duke University, Durham NC, 2005 "Massively Parallel Dip Pen Nanolithography", "Synthetic Supramolecular Allosteric Catalysts." (Mirkin)

228th ACS National Meeting, San Diego, CA, 2005, "Anisotropic Nanostructures: Synthesis, Assembly, and Function," "Bio-barcode Assay: PCR-like Sensitivity for Proteins, Nucleic Acids, and Small Molecules," "Nanoarrays for Probing Fundamental Issues in Nanoscience, Chemistry, and Biology," "Living Templates for the Assembly of Nanoparticle Building Blocks into Functional Architectures." (Mirkin)

AACC Oak Ridge Conference, Baltimore, MD, 2005, "The Bio Barcode Assay: Towards PCR-like Sensitivity for Proteins, Nucleic Acids, Small Molecules, and Metal Ions." (Mirkin)

Princeton University, Princeton, NJ, 2005, "Encoded Nanostructures For Use in Biodiagnostics." (Mirkin)

BioMEMS Seminar, Boston, MA, 2005, "The Bio-Barcode Assay: PCR-like Sensitivity for Proteins, Nucleic acids, and Small Molecules." (Mirkin)

Gordon Research Conference: 3Chem of Supramolecules, Colby College, ME, 2005 "Supramolecular Allosteric Catalysts." (Mirkin)

Gordon Research Conference: Bioorganic Conference, Andover, NH, 2005, "The Bio Barcode Assay: Towards PCR-like Sensitivity for Proteins." (Mirkin)

Gordon Research Conference: Bioorganic Conference, Andover, NH, 2005, "The Bio-Barcode Assay: Towards PCR-like Sensitivity for Proteins." (Mirkin)

BIO2005, Philadelphia, PA, 2005, "Big Academia, A Key to Small Science." (Mirkin)

Gordon Research Conference: Organometallic, Newport RI, 2005, "Allosteric Catalysts Made Possible via the Weak-Link Approach to Supramolecular Coordination Chemistry." (Mirkin)

UCLA ICCOS XVII Conference, Los Angeles, CA, 2005, "The Chemistry and Physical Properties of Self-Organized Nanomaterials." (Mirkin)

MURI Review, Hyattsville, MD, 2005, "Patterning Actuator/Sensor Arrays." (Mirkin)
230th ACS National Meeting, Washington, DC, 2005, "Bio-inspired Assembly of Mesoscopic Building Blocks into Functional Architectures," "New Frontiers in Ultrasensitive Analysis: Nanobiotech, Single Molecule Detection, and Single Cell Analysis," "Bio-programmed Assembly of Nanostructured Materials into Functional Architectures," "Biological Nanoarrays." (Mirkin)

AACI 2005 Annual Meeting, Washington, D.C., 2005, "The Bio-Barcode Assay and Its Implications in Cancer Research." (Mirkin)

Dupont Meeting, Wilmington, DE, 2005, "The Bio-Barcode Assay and Its Implications in Cancer Research." (Mirkin)

MIT Materials Seminar Series, Cambridge, MA, 2005, "Anisotropic Nanostructures: Synthesis Challenges, Assembly, and Biomedical Applications." (Mirkin)

NanoCommerce/SEMI NanoForum, Chicago, IL, 2005, "A Vision for Nanoscience and Nanotechnology." (Mirkin)

Centers of Cancer Nanotechnology Excellence Kickoff Meeting, Bethesda, MD, 2005, "Development of Barcode Assays for Detection of Ovarian Cancer." (Mirkin)

MRS 2005 Fall Meeting, Boston, MA, 2005, "Anisotropic Nanostructures: Synthesis, Assembly, and Function." (Mirkin)

229th ACS Meeting, San Diego, March 2005 (Ratner)

8th European Conference on Molecular Electronics. CNR Area della Ricerca, Bologna, Italy, June-July 2005 (Ratner)

B. Consultative/Advisory Functions

Mirkin consults for Nanosphere and NanoInk.

Ratner collaborates with the Kushmerick and Shashidar groups at NIST and Geo Centers, respectively. Both groups were formerly part of NRL. This collaboration is almost entirely devoted to the vibrational signature of the molecular junction transport. Collaborative research with China Lake Lab on the quite different subject of organic electronics in ongoing.

6. NEW DISCOVERIES, INVENTIONS, OR PATENT DISCLOSURES

A. New Discoveries

Mirkin developed a new technique "On-Wire Lithography" for the fabrication of segmented nanowires consisting of at least two types of materials.

Ratner's findings concerning capacitance and conductance are both of substantial interest, as is his work on vibrational sub-structure of the conductance spectrum.

B. New Inventions and/or Patent Disclosures

"Fabrication of Solid-State Nanostructures Including Sub-50 nm Solid-State Nanostructures Based on Nanolithography and Chemical Etching," Chad A. Mirkin, Hua Zhang, Dana Weinberger, Seunghun Hong

"Fluorophore-Based Bio-Barcode Amplification Assay," Chad A. Mirkin, Jwa-Min Nam, Byung Keun Oh (NU 24084)

"Chemical Release of Particle-Bound Molecules for Molecular Biodetection," Chad A. Mirkin, C. Shad Thaxton, Dimitra Georganopoulou (NU 25002)

"On-Wire Lithography: High Throughput Nanogaps and Nanorod/Disk Arrays," Chad A. Mirkin, Lidong Qin, Sungho Park, Ling Huang (NU 25022)

"A Compact precision XY micropositions for the optical microscope system of commercial DPN writes/AFM (Nscriptor) to locate and monitor one or two-D multiple-probe array over the sample surface," Chad A. Mirkin, Sergey Rozhok (NU 25041)

"Chemically Tailorable Nanoparticles Realized Through Metal-Metalloligand Coordination Chemistry," Chad A. Mirkin, Moonhyun Oh (NU 25042)

"Magnetic gold Nanoparticle Probes Prepared by a Layered Approach "3-Layered Composite Magnetic Nanoprobes for DNA," Chad A. Mirkin, Savka Stoeva, Fengwei Huo, Jae-Seung Lee (NU 25066)

"Generating Nanoarrays of Single Virus Particles by Dip-Pen Nanolithography," Chad A. Mirkin, Rafael Vega, Daniel Maspoch, Khalid Salaita (NU 25088)

"Tailorable hydrophilic surface modification," Chad A. Mirkin, Robert Elghanian (NU NU25103)

"Colorimetric Screening of DNA Intercalators with Gold Nanoparticle Probes," Chad A. Mirkin, Min Su Han, Abigail K.R. Lytton-Jean (NU 25099)

"Surface Enhanced Raman Scattering Substrates from On-Wire Lithography," Chad A. Mirkin, Lidong Qin, Can Xue (NU 25110)

7. HONORS/AWARDS

Mirkin received the NIH Director's Pioneer Award (2004) and the Collegiate Inventors Award, National Inventors Hall of Fame (2005).

Ratner presented a series of named lectures including the Mulliken Medal Lecture at the University of Chicago, the Evans lectures at Ohio State University, and the Lowdin

lectures at the University of Florida. He also received an honorary of Doctorate of Science from the Hebrew University of Jerusalem. Ratner is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, the Royal Danish of Sciences and the International Academy of Quantum Molecular Sciences.